学校编码：10384　　分类号_____ 密级_____
学号：21720061152173　　UDC_____

厦门大学

硕 士 学 位 论 文

巴戟天植株再生体系的建立和花药发育过程的细胞学观察

Establishment of Plant Regeneration System and Cytological Studies on Anther Development of *Morinda officinalis* How

郑 松

指导教师姓名：　田惠桥教授
专 业 名 称：　发育生物学
论文提交日期：　2010 年 4 月
论文答辩日期：　2010 年 6 月
学位授予日期：　2010 年 月

答辩委员会主席：_____
评 阅 人：_____

2010 年 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外,该学位论文为(　　)课题(组)的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

摘 要

巴戟天是一种重要的传统中药材，具有强筋骨、补肾助阳、增强免疫的功效，具有较高的经济价值。利用幼胚离体诱导技术，建立了巴戟天离体培养植株再生体系，为巴戟天的生物技术操作研究奠定了基础。应用组织化学方法，对巴戟天有性生殖中花药发育进行了观察，探讨其花药发育中的营养物质代谢特征。我们的研究结果为巴戟天品种改良打下良好基础。

在巴戟天组织培养中，利用巴戟天的幼胚作外植体，进行快速繁殖和组织培养研究。结果表明： MS + 1.0 mg/L 6-BA + 0.2 mg/L NAA 的培养基适合快速繁殖，再生苗的频率可以达到 100%。将幼胚萌发后的下胚轴切段，转接到 MS + 2.0 mg/L 2,4-D + 0.2 mg/L 或 0.5 mg/L 6-BA 培养基上可以诱导出愈伤组织。将愈伤组织转移到 MS + 1.0 mg/L 6-BA 的培养基中可诱导愈伤组织分化出芽，进而在 1/2 MS + 1.0 mg/L IBA 培养基上诱导生根，再生植株。

对巴戟天花药发育的组织化学观察发现多糖和脂滴类物质的分布呈现一定的规律：在减数分裂之前，花药壁的绒毡层细胞中有少量脂滴，其他细胞中脂滴和淀粉粒都很少。在四分体时期，四分体小孢子中开始出现脂滴，绒毡层细胞中的脂滴较以前增加，其他细胞中的脂滴和淀粉粒仍然很少。在小孢子早期，游离小孢子在其表面形成了花粉外壁，靠外壁下方有一层周缘分布的多糖物质。绒毡层细胞中的脂滴明显减少。发育晚期的小孢子中形成一个大液泡，在细胞质中出现淀粉粒；同时在药壁和药隔组织中也出现了淀粉粒。此时绒毡层退化。在二胞花粉早期，花粉中积累了大量的淀粉粒和一些脂滴。但在成熟花粉中（二胞花粉晚期），淀粉粒消失，只有一定数量的脂滴保留。巴戟天成熟花粉中积累的营养物质主要为脂滴。在巴戟天花药发育过程中，以花粉粒为中心的营养物质输送体系表现出时、空特异性。

关键词：巴戟天 植株再生 花药发育 细胞学观察

Abstract

Morinda officinalis How is an important traditional Chinese herbal medicine, with the function of strengthening the bones and kidneys and enhancing the immune system. And it has high economic value. We established the plant regeneration system of *Morinda officinalis* How with the technique of immature embryo induction *in vitro*, which lay a foundation for biotechnology research of *Morinda officinalis* How. We observed the development of anthers of *Morinda officinalis* How with histochemical methods and investigated its characteristics of nutrient metabolism . These results lay a good foundation for the variety improvement of *Morinda officinalis* How.

We study on rapid propagation and tissue culture of *Morinda officinalis* How with the immature embryo. The results indicate that the culture medium of MS + 1.0 mg/L 6-BA + 0.2 mg/L NAA germination rate up to 100%. The calluses can be induced with hypocotyls in the mediums of MS + 2.0 mg/L 2,4-D + 0.2 mg/L or 0.5 mg/L 6-BA. Calluses were tansferred to the medium of MS + 1.0 mg/L 6-BA, and the buds grow out. The roots are induced in the medium of 1/2 MS + 1.0 mg/L IBA. Whole plants have been successfully regenerated.

We found the distribution of polysaccharides and lipids have certain regularity during the development of *Morinda officinalis* How's anther with the histochemical observation. There were a few lipid drops in the sporogenous cells in young anthers and no starches in the anthers. Before the meiosis of microspore mother cells, some lipids appeared in tapetal cells. The size of tapetal cells began to increase at this stage. At the stage of tetrad, the lipids in the tapetal cells increased, and many lipid drops were accumulated in the cells. Some lipids also appeared in tetrad microspores at this time. There were still no starches in young anther, and only cell wall of anther wall and callose wall of tetrads displayed the feature of polysaccharids. During microspore development, the lipids in tapetal cells decreased evidently. The lipids in the young microspore also disappeared. There were still no starches in anther. At late microspore

stage, some starches accumulated in microspore and appeared in anther wall and connective cells. Tapetal cells degenerated at this stage and its lipid drops concentrated to form lipid block. At early stage of 2-celled pollen, the vegetative cell accumulated a large number of starches. Then the starches in 2-celled pollen disappeared with pollen development, and many lipids were accumulated in mature pollen. Nutrients transport system showed the time and space specificity during the development of *Morinda officinalis* How's anther.

Key words: *Morinda officinalis* How; Plant Regeneration; Anther Development; Cytological Observation

目 录

摘 要 ..... Ⅰ

Abstract ..... Ⅲ

目 录 ..... Ⅴ

Table of Contents ..... Ⅸ

第一章 前言 **1**

1.1 巴戟天研究进展 **1**

1.1.1 巴戟天概述 1

1.1.2 巴戟天栽培研究 4

1.1.3 巴戟天化学成分研究 6

1.1.4 巴戟天药理作用研究 8

1.2 被子植物花药的发育过程 **9**

1.2.1 小孢子发生和雄配子体发育 11

1.2.2 花药壁的发育 13

1.3 研究目的 **16**

第二章 巴戟天离体培养植株再生体系的建立 **17**

2.1 引言 **17**

2.2 实验材料 **17**

2.3 实验试剂及配制 **18**

2.3.1 基本培养基的配制 18

2.3.2 植物激素及其配制 19

2.3.3 常用消毒液、酸碱及其配制 19

2.4 仪器与设备 **19**

2.5 实验方法 **19**

2.5.1 技术路线 19

2.5.2 培养条件.....20
2.5.3 巴戟天组织培养.....20
2.5.4 巴戟天快速繁殖.....21
2.6 结果.....21
2.6.1 巴戟天组织培养.....21
2.6.2 巴戟天快速繁殖.....24
2.7 讨论.....25
2.8 小结.....26
第三章 巴戟天花药发育过程的细胞学观察.....27
3.1 引言.....27
3.2 实验材料.....27
3.3 实验试剂及配制.....28
3.3.1 材料固定相关试剂.....28
3.3.2 组织化学染色相关试剂.....29
3.4 仪器与设备.....30
3.5 实验方法.....30
3.5.1 组织化学染色实验流程.....30
3.5.2 显示多糖、脂类的原理和方法.....32
3.6 结果.....33
3.6.1 造孢细胞时期.....33
3.6.2 小孢子母细胞时期.....33
3.6.3 四分体时期.....34
3.6.4 小孢子早期.....34
3.6.5 小孢子晚期.....34
3.6.6 二胞花粉早期.....35
3.6.7 成熟花粉时期.....35
3.7 讨论.....35
3.8 小结.....37
第四章 创新.....38
缩略词.....39

参考文献..... 40

图版..... 50

硕士研究生阶段发表的论文 54

致谢..... 55

Table of Contents

Abstract in Chinese ..... Ⅰ

Abstract in English ..... Ⅲ

Table of contents in Chinese ..... Ⅴ

Table of contents in English ..... Ⅸ

Chapter 1 Preface 1

1.1 Research progresses of *Morinda officinalis* How 1

1.1.1 The Overview of *Morinda officinalis* How 1

1.1.2 Study on cultivation of *Morinda officinalis* How 4

1.1.3 Study on chemical constituents from *Morinda officinalis* How 6

1.1.4 Study on pharmacological effects from *Morinda officinalis* How 8

1.2 The anther development process of angiosperm 9

1.2.1 Development of microsporogenesis and male gametophyte 11

1.2.2 Development of anther wall 13

1.3 Objective 16

Chapter 2 Establishment of Plant Regeneration System *in Vitro* Culture of *Morinda officinalis* How 17

2.1 Introduction 17

2.2 Materials 17

2.3 Reagents and preparation 18

2.3.1 Preparation of medium 18

2.3.2 Preparation of phytohormone 19

2.3.3 Preparation of the common disinfectant solution,acid and alkali solution 19

2.4 Apparatus and equipments 19

2.5 Methods 19

2.5.1 Technical route 19
2.5.2 Cultural condition 20
2.5.3 Tissue culture of *Morinda officinalis* How 20
2.5.4 Rapid propagation of *Morinda officinalis* How 21
2.6 Results 21
2.6.1 Tissue culture of *Morinda officinalis* How 21
2.6.2 Rapid propagation of *Morinda officinalis* How 24
2.7 Discussion 25
2.8 Conclusion 26
Chapter 3 Cytological Studies on Anther Development of *Morinda officinalis* How 27
3.1 Introduction 27
3.2 Materials 27
3.3 Reagents and preparation 28
3.3.1 Reagents of fixation materials 28
3.3.2 Reagents of histochemical staining 29
3.4 Apparatus and equipments 30
3.5 Methods 30
3.5.1 Experiment process of histochemical staining 30
3.5.2 Principles and methods of polysaccharides and lipids' location 32
3.6 Results 33
3.6.1 The stage of sporogenous cell 33
3.6.2 The stage of microspore mother cell 33
3.6.3 The stage of tetrad 34
3.6.4 The stage of early microspore 34
3.6.5 The stage of late microspore 34
3.6.6 The stage of early bicellular pollens 35
3.6.7 The stage of mature pollens 35
3.7 Discussion 35
3.8 Conclusion 37
Chapter 4 Innovation 38